# Ultrastructural and X-Ray microanalysis of apoptotic cells U-937

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Cell culture U-937 is a convenient model for studying the “extinction” of cellular functions in experiment because these cells can be easily transformed in apoptotic state with different agents. Simultaneous study of functional and morphological changes in cultured cells treated with hypertonic shock and etoposide are revealing disturbances in ion homeostasis in the cells and structural rearrangements in intracellular organelles, such as mitochondria (M), endoplasmic reticulum (ER), Golgi apparatus (GA), cytoskeletal elements, and the ubiquitin-proteasome complex. The results obtained on ultrastructural changes in U-937 cells in the apoptotic state are largely consistent with analogous data available in the literature (Biggiogera et al., 2004) (Fig. 1, 2). One of the important characteristics of apoptotic cells on different stages of apoptosis is thepresence of aggregates of thin osmiophilic particles in the nucleus and the cytoplasm (Fig. 3). The aggregates are not limited by membranes and have varying density and size. Small rod-shaped particles approximately 12 X 30 nm in size are dominated in the aggregates. Immunocytochemical analysis of these cells with polyclonal Abs against α7 subunit of proteasomal protein and colloidal gold (10 nm) have shown that these particles are proteasomes (Fig. 4, 5).

Aggregates of osmiophilic particles have been described in literature in cells situated under extremal conditions: overheating, hypertension, hypoxia, reactive oxygen species, viruses, apoptosis state (Biggiogera et al., 2004; Snigirevskaya et al., 2012). Besides a spontaneous apoptosis associated with the differentiation of mammalian cells and tissues takes place in nature. For example, such processes are described during spermiogenesis and hibernation of animals (Mosevitsky et al., 2012; Biggiogera et al., 2004).

It is generally accepted that these aggregates are formed under conditions of suppressed transcription in the nucleus and involved in the storage and degradation of various mRNA, RNPs and misfolded and overexpressed proteins that should be eliminated by proteolysis using the ubiquitin-proteasome system. In our work we have visualized with electron microscopy one component of this system – proteasomes. Their presence is an indirect confirmation of the involvement of protein ubiquitin in these processes, because these two components operate in tandem.

The results of X-RMA of elemental composition of cells obtained in this study are consistent with the results of other studies (Arrebola et al., 2005). Despite the considerable number of studies devoted to the analysis of changes in the intracellular ions during apoptosis we have revealed changes in the intracellular contents of $Na^+$ and $K^+$ at the level of single cells during apoptosis induced by osmotic shock. An increased ratio of intracellular $Na^+/K^+$ compared to the control for the majority of cells in apoptosis has been shown (Fig.6).

Literature

Arrebola F.,Canizares J., Cubero M.A., Crespo P.V., Warley A., Fernandez-Segura. 2005. *Apoptosis, vol. 10, pp. 1317-1333*.

Biggiogera M., Scovassi A.I., Soldani C., Vecchio L., Pellicciari C. 2004. *Biol. Cell, vol. 96, pp.603-615.*

Mosevitsky M/I/. Snigirevskaya E.S., Komissarchik Ya.Yu. 2012. *Acta Histochem., vol. 114, pp. 237-243.*

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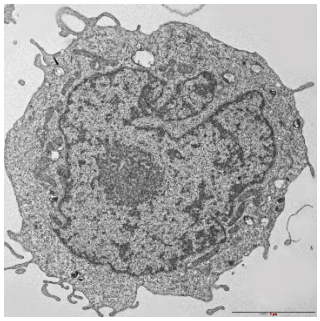
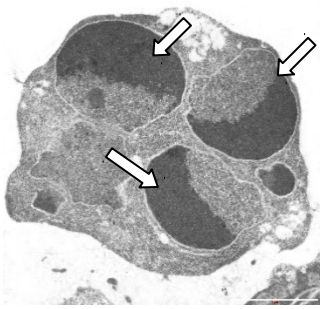
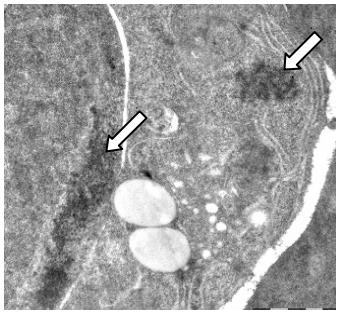


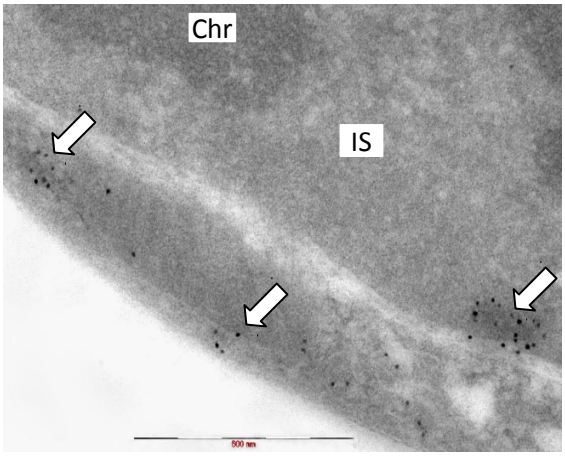
1. Control cell U-937 (Embedding in Epon) N-nucleus, Nu – nucleolus)

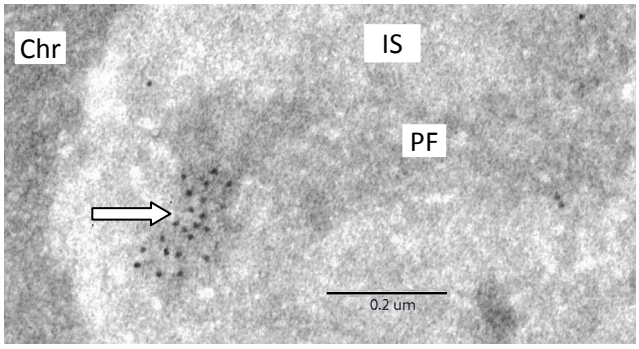


2. Nucleus fragmentation and crescent-like chromatin (arrows) (Etoposide, LR-White)

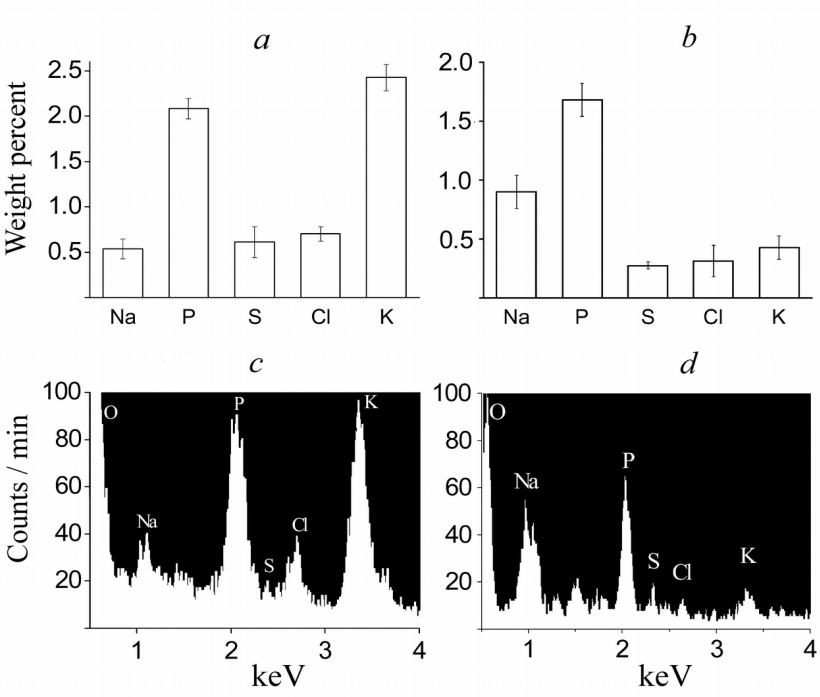


3. Aggregates in the Nucleus and in Cytoplasm (arrows)




4 and 5. Aggregates of proteasomes (arrows) labelled with colloidal gold particles (particle size – 10 nm). On the right photo the perichromatin fibers (PF) are seen inside the interchromatin space (IS) (embedding in LR-White)




6. Elemental composition of control and apoptotic cells U-937 incubated for 4 h in RPMI medium containing 200 mM sucrose. The contents of elements is expressed in weight per cents. a – histogram was constructed on the basis of analyses of X-ray spectra of five control cells. The Na/K ratio is 0,2. b – The histogram was constructed on the basis of X-ray spectra of five apoptotic cells. The Na/K ratio was 2.0. c – X-ray spectrum of a single control cell. d – X-ray spectrum of a single apoptotic cell. The spectra were collected at accelerating voltage of 80 kV for 300s.